Amendments to the Specification

On page 7, please replace paragraph [0032] with the following:

[0032] In yet another aspect of the invention, a method of identifying one or more multimeric proteins having a desired above-threshold activity is provided. The method includes producing a library a bacteriophage or phagemids, each carrying on its outer surface, one of a plurality of different-sequence polypeptides. The different-sequence polypeptides ~~inlcude~~include one of a plurality of first different-sequence heterologous polypeptide segments, one of a plurality of a second different-sequence heterologous polypeptide segments, and joining the two segments, a peptide linker that has a cleavable peptide sequence that is not found in either of said polypeptide segments, and is recognized as a protein cleavage site by a proteolytic enzyme encountered in a bacteriophage host during bacteriophage biogenesis. Cleavage of the linker by the host proteolytic enzyme results in a ~~multimemric~~multimeric protein on the surface of a bacteriophage, each protein (i) having a plurality of different-sequence first and second polypeptides, and (ii) a protein activity related to the sequences of the first and second polypeptides. Bacteriophage in the library that have the above-threshold activity are identified.

On page 9, please replace paragraph [0039] with the following:

[0039] In another embodiment, the first polypeptide segment is N-terminal to the second polypeptide segment, the second polypeptide segment is N-terminal to the third polypeptide segment, and the vector segment encoding the third polypeptide segment further includes one or more ~~suppressable~~suppressible nonsense codon(s) N-terminal to the anchoring segment.

On page 10, please replace paragraph [0050] with the following:

[0050] In one embodiment, the cell is a bacterial cell. The bacterial cell may be selected from the group consisting of strains of Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Klebsiella ~~pneumonial~~pneumoniae, Neisseria gonorrhoeae, and Bacillus subtilis.

On page 13, please replace paragraph [0072] with the following:

[0072] A typical antibody structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively. The variable region of the heavy or light chain typically comprises four framework regions each containing relatively lower degrees of ~~variablity~~variability that includes lengths of conserved sequences. Framework regions are typically conserved across several or all immunoglobulin types and thus conserved sequences contained therein are particularly suited for preparing repertoires having several immunoglobulin types.

On page 17, please replace paragraph [0088] with the following:

[0088] As used herein, the term “wild-type” refers to a gene or gene product which has the characteristics of that gene or gene product when isolated from a naturally occurring source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designated the normal or wild-type form of the gene. In contrast, the term “modified” or “mutant” ~~referes~~refers to a gene or gene product which displays modifications in sequence and/or functional properties, i.e., altered characteristics, when compared to the wild-type gene or gene product.

On page 22, please replace paragraph [00113] with the following:

[00113] The number of possible combinations of heavy and light chains may exceed 10^{12}. To sample as many combinations as possible depends, in part, on the ability to recover large numbers of ~~tranformants~~transformants. For phage with plasmid-like forms, e.g., filamentous phage, electrotransformation provides an efficiency comparable to that of phage-transfection with *in vitro* packaging, in

addition to a very high capacity for DNA input. This allows large amounts of vector DNA to be used to obtain very large numbers of transformants. The method described by Dower *et al.* (1988) *Nucleic Acids Res.*, 16:6127-6145, for example, may be used to transform fd-tet derived recombinants at a rate of about 10^7 transformants/μg of ligated vector into *E. coli*, and libraries may be constructed in fd-tet B1 of up to about $3x10^8$ members or more.

On page 24, please replace paragraph [00117] with the following:

[00117} A related embodiment of the invention, as illustrated in Fig. 2, follows the same principles as above, except that the cleavable linker 37 is designed to be cleaved by a cytoplasmic protease (either endogenous or exogenous, as described in Section IA below), and an additional signal peptide 33 is included upstream of the third polypeptide 34. Fig. 2 shows the multimeric protein encoded by the fusion gene, before cleavage and after cleavage of the leader sequence 30 and the signal peptide 33 at the leader cleavage sites 31 and 39 and the linker cleavage site 37, translocation and dimeric assembly. Again, preferably, the anchoring peptide sequence 35 is a phage coat protein; the first polypeptide segment 32 is a light chain; and the third polypeptide segment 34 is the variable and CH1 domain of a heavy chain. Optionally, a linker 38, which may be cleavable as described herein, links third polypeptide segments 34 to the anchoring peptide sequence 35. Alternatively, the positions of the heavy and light chains are reversed. Thus, a dimeric polypeptide 40 is assembled as a Fab fragment anchored to a coat protein 42. Optionally, a linker 78, which may be cleavable as described herein, links third polypeptide segments 74 to the anchoring peptide sequence 75. Optionally, a covalent bond 36 links the first 32 and third 34 polypeptide segments, respectively.

On page 26, please replace paragraph [00126] with the following:

[00126] Inteins are described in, for example, U.S. Pat. Nos. 5,981,182, and 5,834,247, which are herein incorporate by reference in their entirety for all purposes and for the purpose of teaching inteins and intein chemistry. Inteins

generally include amino acid residues that are conserved among inteins of different proteins. Intein motifs are described in, for example, Pietrokovski, S. (1994) *Protein Science* 3:2340-2350; Perler et al. (1997) *Nuc. Acids Res.* 25:1087-93; Pietrokovski, S. (1998) *Protein Sci.* 7:64-71. Other methods of identifying inteins are described in, for example, Dalgaard et al. (1997) *J. Computational Biol.* 4:193-214 and Gorbalenya, A. E. (1998) *Nucleic Acids Res* 26:1741-8. "INBASE" a compilation of known inteins by New England Biolabs, is found at ~~http://~~circuit.neb.com/inteins/int_id.html.

On page 28, please replace paragraph [00131] with the following:

[00131] In one embodiment of the invention, the link between the antibody chains is sufficiently short, e.g. less than 10 amino acids, such that the two chains cannot associate together until the link is cut. The result of this may be that a folded monomeric single chain Fab is not produced as a transient product. This embodiment is schematized in Figs. 4A and 4B, where the polypeptide segments dimerize to form a dimeric molecule with two potential binding sites. A vector segment encodes two polypeptide sequences 79, each of which include a first polypeptide segment 72, a second polypeptide segment, not shown, or linker 77, and a third polypeptide segment 74 having an anchoring peptide sequence 75 for anchoring the multimeric polypeptide to the surface of a genetically replicable package. Optionally, a linker 78, which may be cleavable as described herein, links third polypeptide segments 74 to the anchoring peptide sequence 75. The dashed vertical line 76 represents a disulfide bond that covalently links the first and third polypeptide segments 72 and 74, respectively. A leader sequence 70 that is cleaved at point 71 is also shown.

On page 28, please replace paragraph [00134] with the following:

[00134] It should be appreciated that the above-described embodiments ~~refering~~referring to polypeptides as first, second or third polypeptide segments may be oriented in either a N-terminal to C-terminal direction, or vice-versa. Thus, the

first polypeptide segment may be at either the N-terminus or C-terminus of the polypeptide. Likewise, the third polypeptide, and/or the anchoring peptide segment, may be positioned at either the N-terminus or C-terminus of the polypeptide.

On page 30, please replace paragraph [00137] with the following:

[00137] In another embodiment of the invention, as illustrated in Fig. 3A, the link 57 between the first polypeptide segment 52 and third polypeptide segment 54 is sufficiently long to form a single-chain Fab polypeptide 60, anchored to segment 62. An anchoring peptide sequence 55 is linked to the third polypeptide segment 54 by a linker 58, which optionally may be cleavable. Linker 57 is preferably cleaved as described herein and illustrated in Fig. 3B. Below the arrow in Fig. 3A, the processed and folded single-chain Fab fragment 60 is shown anchored to the phage coat protein 55. The dashed vertical line 56 represents the disulfide bond that covalently links the first and third polypeptide segments 52 and 54, respectively. Preferably, the first and third polypeptide segments are an antibody light and heavy chains. The anchoring peptide sequence 55 is preferably a phage coat protein, e.g. gpIII or gpVIII. Similar to Fig. 1, Figs. 3A-3B show a leader sequence 50 that is cleaved at point 51.

On page 31, please replace paragraph [00142] with the following:

[00142] The multimeric polypeptide may be a wide variety of substances in addition to antibodies. These include, e.g., growth factors, hormones, enzymes, interferons, interleukins, intracellular and intercellular messengers, lectins, cellular adhesion molecules and the like. See, e.g., U.S. Patent No. 6,291,160, which is incorporated by reference herein. Ligands corresponding to these ~~mulitmeric~~multimeric polypeptides can also be identified. Thus, although antibodies are widely available and conveniently manipulated, they are merely representative of the multimeric polypeptides of the present invention.

On page 34, please replace paragraph [00152] with the following:

[00152] For a given bacteriophage, the preferred means for displaying the multimeric protein is with the use of a protein that is present on the phage surface, e.g. a coat protein. Filamentous phage can be described by a helical lattice; isometric phage, by an icosahedral lattice. Each monomer of each major coat protein exists on a lattice point and makes defined interactions with each of its neighbors. Proteins that fit into the lattice by making some, but not all, of the normal lattice contacts are likely to destabilize the virion by aborting formation of the virion as well as by leaving gaps in the virion so that the nucleic acid is not protected. Thus, in bacteriophage, unlike the cases of bacteria and spores, it is generally important to retain in the antibody fusion proteins those residues of ~~hte~~the coat protein that interact with other proteins in the virion. For example, when using the M13 cpVIII protein, the entire mature protein will generally be retained with the antibody fragment being added to the N-terminus of cpVIII, while on the other hand it can suffice to retain only the last 100 or fewer carboxy-terminal residues of the M13 cpIII coat protein in the multimeric protein fusion.

On page 35, please replace paragraph [00154] with the following:

[00154] To enrich and isolate phage that encode a selected multimeric polypeptide, and thus to ultimately isolate the nucleic acid sequences themselves, phage harvested from the bacterial debris are affinity-purified. As described below, when a multimeric polypeptide which specifically binds a particular target is desired, the target may be used ~~ot~~to retrieve phage displaying the desired multimeric polypeptide. The phage so obtained may then be amplified by infecting into host cells. Additional rounds of affinity enrichment followed by amplification may be employed until the desired level of enrichment is reached.

On page 36, please replace paragraph [00158] with the following:

[00158] The mature capsule of Ff phage is comprised of a coat of five phage-encoded gene products: cpVIII, the major coat protein product of gene VIII that

forms the bulk of the capsule; and four minor coat proteins, cpIII and cpIV at one end of the capsule and cpVII and cpIX at the other end of the capsule. The length of the capsule is formed by 2500 to 3000 copies of cpVIII in an ordered helix array that forms the characteristic filament ~~sturcture~~structure. The gene III-encoded protein (cpIII) is typically present in 4 to 6 copies at one end of the capsule and serves as the receptor for binding of the phage to its bacterial host in the initial phase of infection.

On page 36, please replace paragraph [00161] with the following:

[00161] The 50-amino acid mature gene VIII coat protein (cpVIII) is synthesized as a 73 amino acid precoat. cpVIII has been extensively studied as a model membrane protein because it can integrate into lipid bilayers such as the cell membrane in an asymmetric orientation with the acidic amino terminus toward the outside and the basic carboxy terminus toward the inside of the membrane. The first 23 amino acids constitute a typical signal-sequence that causes the nascent polypeptide to be inserted into the inner cell membrane. An *E. coli* signal peptidase (SP-I) recognizes amino acids 18, 21, and 23, and, to a lesser extent, residue 22, and cuts between residues 23 and 24 of the precoat. In one embodiment of the invention, this sequence is mutated to improve the display of the multimeric protein as described in Jestin, JL *et al.* (2001) *Res. Microbiol.*, Mar;152(2):187-91. After removal of the signal sequence, the amino terminus of the mature coat is located on the periplasmic side of the ~~innter~~inner membrane; the carboxy terminus is on the cytoplasmic side. About 3000 copies of the mature coat protein associate side-by-side in the inner membrane.

On page 38, please replace paragraph [00166] with the following:

[00166] Similar constructions are contemplated for other filamentous phage. Pf3 is a well known filamentous phage that infects ~~*Pseudomonos aerugenosa*~~ *Pseudomonas aeruginosa* cells that harbor an IncP-I plasmid. The entire genome has been sequenced and the genetic signals involved in replication and assembly

and protein interactions during its membrane protein insertion are known (Chen, M *et al.* (2002) *J. Biol. Chem.* 277(10):7670-5). The sequence has charged residues Asp-7, Arg-37, Lys-40, and Phe44 which are consistent with the amino terminus being exposed. Thus, to cause a multimeric polypeptide to appear on the surface of Pf3, a tripartite gene can be constructed which comprises a signal sequence known to cause secretion in *P.* ~~*aerugenosa*~~aeruginosa, fused in-frame to gene fragments encoding a polypeptide sequence that includes a cleavable peptide sequence cleavable by a proteolytic agent, which is fused in-frame to a gene encoding the mature Pf3 coat protein, or fragment thereof. Optionally, DNA encoding a flexible linker of one to ten amino acids is introduced between the polypeptide sequence and the Pf3 coat protein gene. This tripartite gene is introduced into Pf3 so that it does not interfere with expression of any Pf3 genes. Once the signal sequence is cleaved off, the multimeric polypeptide is in the periplasm and the mature coat protein acts as an anchor and phage-assembly signal.

On page 41, please replace paragraph [00173] with the following:

[00173] Among bacterial cells, preferred genetically replicable packages include Salmonella ~~typhirnurium~~typhimurium, Bacillus subtilis, Pseudomonas aeruginosa, Vibrio cholerae, Klebsiella pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Bacteroides nodosus, Moraxella bovis, and especially Escherichia coli. Many bacterial cell surface proteins useful in the present invention have been characterized. See, e.g., Benz et al. (1988) Ann. Rev. Microbiol. 42:259-93; Balduyck et al. (1985) Biol. Chem. Hoppe-Seyler 366:9-14; Ehrmann et al. (1990) PNAS 87:7574-78; Heijne et al. (1990) Protein Engineering 4:109-12; Ladner et al. U.S. Pat. No. 5,223,409; Fuchs et al. (1991) Biotechnology 9:1370-72; and Goward et al. (1992) TIBS 18:136-40.

On page 44, please replace paragraph [00183] with the following:

[00183] Selection based on slow dissociation rate, which is usually predictive of high affinity, is a very practical route. This may be accomplished by increasing the volume, number, and/or length of the washes. In each case, the rebinding of dissociated multimeric polypeptide/package is prevented, and with increasing time, multimeric polypeptide/packages of higher and higher affinity are recovered. Moreover, additional modifications ~~fo~~of the binding and washing procedures may be applied to find multimeric polypeptides with special characteristics. The affinities of some multimeric polypeptides, e.g., antibodies, are dependent on ionic strength or cation concentration. This is a useful characteristic for antibodies to be used in affinity purification of various proteins when gentle conditions for removing the protein from the antibody are required. Specific examples are antibodies which depend on Ca^{++} for binding activity and which lose or gain binding affinity in the presence of EGTA or other metal chelating agent. Such antibodies may be identified in the recombinant antibody library by a double screening technique isolating first those that bind the target in the presence of Ca^{++}, and by subsequently identifying those in this group that fail to bind in the presence of EGTA.

On page 45, please replace paragraph [00187] with the following:

[00187] DNA prepared from eluted phage may be transformed into host cells by electroporation or other well known chemical means to further minimize ay problems associated with defective infectivity. The cells are cultivated for a period of time sufficient for marker expression, and selection is applied as typically performed for DNA ~~tranformation~~transformation. The colonies are amplified, and phage harvested for a subsequence round or rounds of panning.

On page 48, please replace paragraph [00195] with the following:

[00195] Another aspect of the invention provides kits for practice of the methods described herein. The kits preferably include members of a phage display library,

e.g., phage particles, vectors, and/or cells containing phage. The assay kits may additionally include any of the other components described herein for the practice of methods or assays of the invention. Such materials include, but are not limited to, helper phage, or ~~or~~ more bacterial or eukaryotic cell lines, buffers, antibiotics, labels, and the like.